THE EFFECTS OF SYMPATRIC AND ALLOPATRIC HAB SPECIES ON CALANOID COPEPOD SWIMMING BEHAVIOR

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THE EFFECTS OF SYMPATRIC AND ALLOPATRIC HAB SPECIES ON CALANOID COPEPOD SWIMMING BEHAVIOR

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LIST OF SYMBOLS AND ABBREVIATIONS

HAB	Harmful algal bloom
NGDR	Net to gross displacement ratio
V	Swimming velocity
x_t, y_t, z_t	The copepod's position in x, y, and z planes at time t

SUMMARY

Harmful algal blooms, commonly referred to as red tides, occur yearly with dramatic impacts on marine ecology, coastal economies, and human health. As a consequence, research into the zooplankton grazers that consume HABs is highly important. However, changes in ocean temperature may increase the range of many HABs, exposing historically naive copepods to new species and their associated chemicals. Little research into the impact of allopatric verses sympatric species, particularly on the immediate behavioral impact, has been performed, leaving the indirect fitness effects of HAB exposure and consumption relatively unknown. We measured alterations in the swimming behavior of the calanoid copepod *Temora longicornis* following exposure to sympatric *Alexandrium fundyense* and allopatric *Karenia brevis* treatments. After a 15-16 hours depuration period post*A. fundyense* exposure, *T. longicornis* exhibited increased average swimming speed and an elevated net to gross displacement ratio (NGDR). During exposure to*K. brevis*, copepods exhibited an immediate decrease in swimming speed and NGDR, as well as an increased frequency of jump behavior. However, these effects faded after an one-hour depuration period, and disappeared after a 15-16 hour depuration period. The alterations in swimming behavior demonstrated by the copepods treated *A. fundyense* may increase encounter rate with predators, while copepods treated with *K. brevis* remain in bloom conditions for longer periods of time, negatively affecting survivorship. *Temora longicornis* individuals also may be made more visible to predators due to the increase in jumps seen during treatment with *K. brevis.* These behavioral changes suggest how HABs escape from zooplankton grazer control by altering copepod swimming behavior, and the pattern of predator-prey evolution that occurs over time.

CHAPTER 1

INTRODUCTION

Overgrowth of toxic algal species known as harmful algal blooms (HABs) or red tides are an increasingly widespread ecological problem, impacting marine productivity and ecosystem function through multiple trophic levels (Glibert 2005). Both the number and impact of HABs have increased over the previous decades, causing harm in almost all coastal locations to marine and terrestrial organisms alike, including humans (Anderson 1994, 1997; Anderson et al 2010). A factor thought to contribute significantly to the proliferation of blooms is phytoplankton avoidance of zooplankton predator control through a number of methods (Irigoien et al 2005). HAB species produce neurotoxins, associated chemicals, and have limited nutritional value, which may impact copepod fitness and behavior (Irigoien et al. 2005).Much of current research focuses on the physiological impact of HAB species on zooplankton, but focusing research on animal behavior is also important. Behavior experiments show the immediate impact of experimental treatments, even during intermittent exposure periods. In contrast, physiological experiments, such as measuring egg production, hatching success, or survival rates, require extended periods of exposure and yield delayed results. Understanding the impact of toxic algae on zooplankton behavior is important in determining the methods by which algae escape predator control, and may provide an early warning system for the detection of future red tides through development of simple and sensitive behavioral bioassays.

One component that may contribute to the changes in interactions between harmful algae and zooplankton may be biogeography. Historical exposure to HAB species may result in the reduction of negative fitness effects, as grazers that can tolerate HABs will survive, producing

more robust and HAB-resistant offspring (Colin and Dam 2005). Resistance can evolve in copepod populations over the course of a few decades, with selective pressure due to bloom exposure shifting population demographics towards universal resistance (Colin and Dam 2007). Conversely, grazers without historical exposure may be more vulnerable to the effects of neurotoxins, other chemicals, or nutritional depletion. Harmful algae species are known to affectgrazers differently based on species of algae and zooplankton (Cohen et al 2007). *Karenia brevis*, a harmful algae species native to the Gulf of Mexico, had an impact on the mortality and swimming behavior in multiple copepod species, with effects varying in magnitude and manifestation based on grazer species (Cohen et al 2007). Fitness consequences in *Acartia tonsa* fed a rich *K. brevis,* including decreased survivorship and egg production, were due to the nutritional inadequacy of the HAB rather than brevetoxins (Prince et al. 2006). Individual *A. tonsa* limited to *K. brevis* only diet consumed 480% more than individuals fed the palatable algal species *Rhodomonas lens*, likely to combat the low nutritional quality through consumption quantity (Prince et al. 2006). Local adaptation of *Acartia hudsonica* to the harmful algae species *Alexandrium fundyense* also has been observed, with populations of *A. hudsonica* exposed to *A. fundyense* blooms over several decades exhibiting no loss in fitness when compared to historically naive populations (Colin and Dam 2005, 2007). However, comparison of sympatric and allopatric grazer and harmful algal species pairings with Mediterranean species has shown that historical exposure does not always modify the effects of HABs (Turner et al 2012). When *Temora stylifera* was exposed to the sympatric species *Alexandrium tamarense,* significant sublethal effects were observed compared to the allopatric pairing of *Calanus helgolandicus* and *A. tamarense,* even though the strains used experimentally did not produce neurotoxins (Turner et al 2012). Additionally, exposure to one HAB species does not appear to impart physiological

resistance to novel harmful algal species, as *T. stylifera* also was more detrimentally affected by the novel harmful species *K. brevis* compared to *C. helgolandicus,* which does not co-occur with either HAB species (Turner et al 2012).

Although most research into the effects of HABs of zooplankton focus on fitness, mortality, and reproduction, the importance of behavioral responses cannot be overlooked. Copepods are known to screen food sources to avoid incapacitation due to neurotoxins or other HAB produced chemicals, selectively consuming non-saxitoxin producing species or strains with a reduced saxitoxin load (Teegarden 1999, Wagget 2012). Cohen et al (2007) showed that multiple zooplankton species alter swimming behavior in the presence of *K. brevis* through highly variable, but generally increased positive phototaxis, lower percentage of animals observed swimming, and increased variability in swimming speeds.

Many previous studies evaluate the effects of constant exposure to harmful algae over a prolonged period of time, particularly when measuring consumption and sub-lethal fitness effects (Hong et al 2012, Colin and Dam 2007, Waggett 2012).Blooms are known to be patchy depending on a variety of marine conditions, and given the known high search volume rates of copepods, the immediate and delayed behavioral reactions to temporary bloom exposure could vary greatly (Thomas et al 2010, Kiorboe and Bagoien 2005).Integrating the importance of patchiness and intermittent exposure provides insight into the immediate and persistent effects of HABs on zooplankton, which may increase our ability to predict the occurrence of blooms and track the transfer of toxins to higher trophic levels.Patchiness occurs when the concentration of HABs is non-uniform across an environment, and can vary depending on ocean currents, wind, stratification, and nutrient concentration (Glibert et al. 2005). Depuration experiments have been performed primarily in bivalves, with depuration rates ranging from days to months depending

on the species (Landsberg 2002). In copepods such as *A. tonsa* and *Temora longicornis*, chemical uptake and depuration appears to be dose-dependent, with high concentrations of chemical-producing organisms in the experiment environment yielding higher chemical uptake and longer depuration periods (Lincoln et al. 2001). The amount of time copepods spend directly exposed to HABs directly affects the amount of neurotoxins that propagate upwards through the marine food web (Tester et al. 2002).

Behavioral studies of copepods rarely observe the depuration period post HAB exposure. The copepod species *Temora longicornis* does not show an immediate reaction to *A. fundyense* exposure, but exhibits an elevated swimming speed 15-16 hours after removal from a two-hour period of bloom conditions (Lasley-Rasher 2012). The increase in swimming speed results in a 24-54% increase in predator encounter frequency, a mortality aspect not integrated with current models of bloom proliferation and zooplankton biomass (Lasley-Rasher et al 2012, Roelke and Buyukates 2001).

Determining the difference in allopatric vs. sympatric zooplankton/HAB interactions may become more importantdue to the changing geographical range of some HAB species. The HAB species *K. brevis*, native to the Gulf of Mexico, has bloomed off the east Atlantic Coast multiple times, reaching as far as North Carolina in 1987 (Glibert et al. 2005, Tester et al. 1991). The HAB responsible for paralytic shellfish poisoning (PSP) is native to the north Atlantic and Pacific, primarily blooming off the coasts of Maine and Massachusetts on the east coast of the United States and Washington, Oregon, and Northern California in the west coast (Anderson et al. 2010). However, PSP blooms have been recorded much further south in recent decades, invading southern California, New York, Delaware, and Maryland (Anderson et al. 2010). Understanding the impact of an allopatric HAB species on copepod behavior will help predict

the potential damage a novel HAB species may cause on a naive environment.

The two dinoflagellates selected in this study are *A. fundyense* and *K.brevis*, which produce saxitoxins and associated compounds (responsible for paralytic shellfish poisoning) and brevetoxins(responsible for neurotoxic shellfish poisoning) respectively (Lansberg 2002). The intensity, frequency, and geographic range of *Alexandrium* spp. and *K. brevis* blooms have significantly increased in the past 30 years (Anderson et al. 2010, Sellner et al. 2003). *Alexandrium* spp. causes yearly deaths and costs the states of Maine and Massachusetts millions of dollars in shellfish sales when blooms occur (Bienfang et al. 2011). *Karenia brevis* causes shutdowns of coastal waters and fisheries throughout the Gulf of Mexico, impacting fishing and tourism, and can cause severe health problems for people living in the surrounding areas (Bienfang et al. 2011). In early 2013, a *K. brevis* bloom off the west coast of Florida made headlines due to unprecedented deaths in the manatee population, with the HAB responsible for the deaths of 241 of the estimated 5,000 manatees (Wines 2013).

Temora longicornis behavior is known to be altered by exposure to *A. fundyense*, and the behavior of multiple copepod species were affected by treatments of *K. brevis*, fueling the hypothesis that treatments of *A. fundyense* and *K. brevis* will result in measurable behavior changes during these experiments (Lasley-Rasher 2012, Cohen et al. 2007, Turner et al 2012). Previous research in the physiological and behavioral reaction of copepods to different HAB species suggests that an allopatric HAB-copepod pairing will result in greater negative fitness implications than a sympatric pairing. In addition, observations of copepod behavior during the depuration period will likely differ from observations during copepod exposure to HAB treatments due to the presence or absence of HAB-produced chemicals and nutritional deficiencies.

This study addresses the following questions: 1) Does the calanoid copepod *Temora longicornis* exhibit changes in swimming behavior when exposed to *A. fundyense* and *K. brevis*? 2) Do any behavioral effects persist immediately after removal from a bloom and 15-16 hours after bloom exposure? 3) Are there any differences in behavioral alterations in a sympatric bloom (*A. fundyense*) compared to an allopatric bloom (*K. brevis*)?

CHAPTER 2

METHODS

Organism collection and culture. The target calanoid copepod species *Temora longicornis* occurs throughout the North Atlantic Ocean. Experimental copepods were collected from Walpole, Maine (43˚56' N, 69˚35' W), specifically the Damariscotta River estuary. Animals were collected using a 250µm mesh plankton net towed by boat obliquely at an approximate depth of 30m during spring 2009-2012. The collected organisms were transferred to 20L containers and moved to a temperature-controlled location before shipment overnight to the Georgia Institute of Technology. Animals and water were sealed in 1-2L polyethylene containers sealed with parafilm to minimize the impact of bubbles during shipment. Bottles were packed with ice packs to control temperature and cushioning material to prevent damage. Upon arrival at Georgia Tech, bottles were removed from shipping boxes and placed in a temperature-controlled room (10-14°C) to acclimate. After an adjustment period, animals were transferred into 20L containers, diluted with artificial seawater, and fed a mixture of *Tetraselmis* spp. and *Isochrysis galbana*. Animals were allowed a 24-hour adjustment period before *T. longicornis* adults were sorted under a dissecting microscope. Sorted copepods were moved to separate containers at densities of less than 25 animals per liter of filtered artificial seawater.

The algal species *Rhodomonas lens* was selected as the control species due to its common use in culture, non-toxic nature, and distribution throughout the Atlantic and Gulf of Mexico (Koski et al. 1998). The sympatric harmful algal species selected for study was *Alexandrium fundyense*, known to bloom yearly in the Gulf of Maine and produce the saxitoxin responsible for paralytic shellfish toxin (PST)(Anderson et al. 2010). *Karenia brevis*, native to the Gulf of

Mexico, was selected as the allopatric HAB species due to its isolation from *T. longicornis* and production of the brevetoxin responsible for neurotoxic shellfish toxin (Cohen et al. 2007). *Tetraselmis* spp. and *I. galbana* are commonly used in zooplankton culture, and *Tetraselmis* spp. used in previous experiments during the depuration period post HAB exposure (Breteler and Gonzales 1986, Dam and Haley 2011). Phytoplankton cultures were kept at controlled temperatures (21°C for *R. lens, K. brevis, Tetraselmis* spp., and *I. galbana;* 14°C for *A. fundyense*) at a 14:10 light:dark cycle.

Table 1. Phytoplankton characteristics

Species	Strain	Shape	Dimensions (mean ± s.e. µm)	Replicates (n)	Cell biovolume (mean ± s.e. μm^3)
R. lens	NCMA 739	Rotational ellipsoid	13.5 ± 1.8 by 6.9 ± 0.3	7	$3.36x10^2$
A. fundyense	NCMA 1719	Rotational ellipsoid	26.6 ± 1.9 by 21.8 ± 0.5	12	$6.61x10^3$
K. brevis	NCMA 2228	Flattened ellipsoid	31.9 ± 2.6 by 22.6 ± 3.1 by 16.18 ± 0.95	12	$6.60x10^3$

Toxin analysis. The *A. fundyense* and *K. brevis* strains used in these experiments were confirmed toxic through ELISA (enzyme-linked immunosorbant assay). Testing of the *A. fundyense* samples was done by Greenwater Laboratories in Palatka, Florida, and the *K. brevis* samples were tested internally in Dr. Julia Kubanek's lab at Georgia Tech (Naar et al. 2002). The ELISA tests indicated the presence of saxitoxin and brevetoxin respectively.

Treatments. Four treatments were used in the behavior experiments: two control treatments and two HAB treatments. All treatment concentrations were determined using the biovolume of a 100% *K. brevis* medium concentration bloom (Turner 1997). The first *R. lens* control (100%) had a concentration of 5600 cells/mL to test copepod behavior in a completely

palatable bloom environment. The second *R. lens* (20%) control had a concentration of 1120 cells/mL (20% biovolume equivalent to the *K. brevis* medium concentration bloom) to test copepod behavior at the reduced food levels present in the HAB treatments. The first HAB treatment had an 80% *K. brevis* (320 cells/mL) and 20% *R. lens* (1120 cells/mL) mixture to test copepod swimming behavior in the presence of *K. brevis* bloom concentrations. The second HAB treatment had an 80% *A. fundyense* (320 cells/mL) and 20% *R. lens* (1120 cells/mL) mixture to test copepod behavior in the presence of *A. fundyense* bloom conditions. The inclusion *R. lens* in the HAB treatments was used to mimic natural bloom conditions and to ensure the constant presence of palatable food throughout the experiments. Table 2 details the terms that are used throughout the results and discussion to refer to the treatments.

Behavior Experiments. The behavior experiments were performed to determine if the swimming behavior of *T. longicornis* was affected by exposure to the *A. fundyense* and *K. brevis* treatments. Copepods were separated by sex at least 24 hours prior to experiments. Fifteen male and 15 female copepods were place in a 1L tank containing 800mL of filtered artificial seawater, exposed to one of the control or HAB treatments at random, then placed in the temperature controlled (10-14°C) observation tank. All experiments were visualized using a Schlieren optical system during the spring season (2009-2012) to eliminate seasonality as a variable, and at least one experiment from each treatment type was performed each year. Four replicate trials using different *T. longicornis* individuals from stock cultures were performed for each treatment. Swimming behavior experiments were recorded in complete darkness, with a single green laser directed down the center of each tank to attract animals to the center, increasing the number of visible interactions and reducing the limiting effects of the tank walls (Doall et al. 1998). The green laser was included in all experimental hours and replicates to ensure variations in behavior

due to its presence were minimized.

The 30 copepods remained in the tank with the HAB or control treatments for two hours, a one-hour acclimation period (Treatment hour 0), which was not used for data collection, and a second hour for data collection (Treatment hour 1). At the end of the two hour treatment period, animals were removed from the treatment and placed into a clean 1L tank containing 800mL filtered artificial seawater and a saturated level of *Tetraselmis* spp ($2.5x10^4$ cells/mL), beginning the depuration period (Buttino et al. 2009). The tank was return to the observation vessel and observed for one hour (Depuration hour 0), after which the tank was removed, covered with parafilm, and placed in a temperature-controlled incubator overnight at 10-14°C. Following 15 hours of depuration, the tank was returned to the observation vessel and the copepod swimming behavior recorded for the final experimental hour (Depuration hour 15). *T. longicornis* individuals exposed to the *R. lens* controls were returned to the unsorted stock cultures and were not used in subsequent experiments. Individuals exposed to the *A. fundyense* and *K. brevis* treatments were discarded to ensure no transfer of either HAB species into the stock culture.

Table 2. Experimental terms and descriptions

Term	**Description**
R. lens control (100%)	The full bloom biovolume *R. lens* control, 5600 cells/mL
R. lens control (20%)	The reduced bloom biovolume *R. lens* control, 1120 cells/mL
R. lens control	The combined data from the two R. lens controls (100% and 20%), when statistically appropriate
A. fundyense treatment	The sympatric HAB treatment, 80% *A. fundyense* (320 cells/mL) and 20% *R. lens* (1120 cells/mL)
K. brevis treatment	The allopatric HAB treatment, 80% *K. brevis* (320 cells/mL) and 20% *R. lens* (1120 cells/mL)
Treatment hour 0 (T0)	The first hour (0-1) of copepod exposure to control or HAB treatments, acclimation period
Treatment hour 1 (T1)	The second hour (1-2) of copepod exposure to control or HAB treatments, first hour of data

	collection
Depuration hour 0 (D0)	The first hour (0-1) after copepod removal from HAB or control treatments and placement in clean water + *Tetraselmis*
Depuration hour 15 (D15)	The 15-16th hour (overnight) after copepod removal from HAB or control treatments and placement in clean water + *Tetraselmis*

Behavior tracking and analysis. All swimming behavior observations were recorded onto DVDs, which were subsequently digitized and cut into 10-20 second clips for analysis. SolveigMM Video Splitter or Avidemux Video Editor were used to clip the tapes, and Prism Video Converter was used to convert all files into the appropriate format (.avi) for the tracking programs. Certain tapes of poor visual quality were edited to enhance brightness and contrast in Adobe After Effects CS5 (Adobe), although no alterations were made to any other settings or the clip content. The calibration clips associated with any videos subjected to editing were also editing using the same technique to ensure the fidelity of the data. Edited and unedited clips were analyzed using either the LabTrack software (Bioras) or the Hedricks software for Matlab (Hedrick 2008). LabTrack was used as the default tracking program, while the Hedricks software was only used in cases where the visual quality was too poor for successful tracking in LabTrack. Both programs track individual movement on a frame-by-frame basis, recording the X and Y pixel coordinates of each individual over time, cataloging the trajectories in spreadsheet formats (.csv).

The three-dimensional movement of the freely-swimmingcopepods was obtained by tracking images produced in a Schlieren-based videography system that combines two 2D images of orthogonal views of the same event onto a single camera image (Figure 1, see also Strickler 1998; Doall et al. 1998). To distinguish the x-z_1 location of the copepod from its y-z_2

location, the mirrors are slightly offset so that z_1 is slightly lower, and therefore always associated with the x coordinate. The X and Y coordinates are taken directly from the pair of trajectories, and the Z coordinates of each orthogonal image are averaged (Figure 1). A 1 cm calibration stick, placed in the tank at the beginning of each experimental hour, was used to convert the X and Y pixel coordinates to metric measurements. Several criteria were used to determine a viable path: copepods had to be swimming in or near the center of the tank (producing orthogonal image pairs to obtain the 3D trajectory), and tracks had to last ≤10 seconds (300 frames).

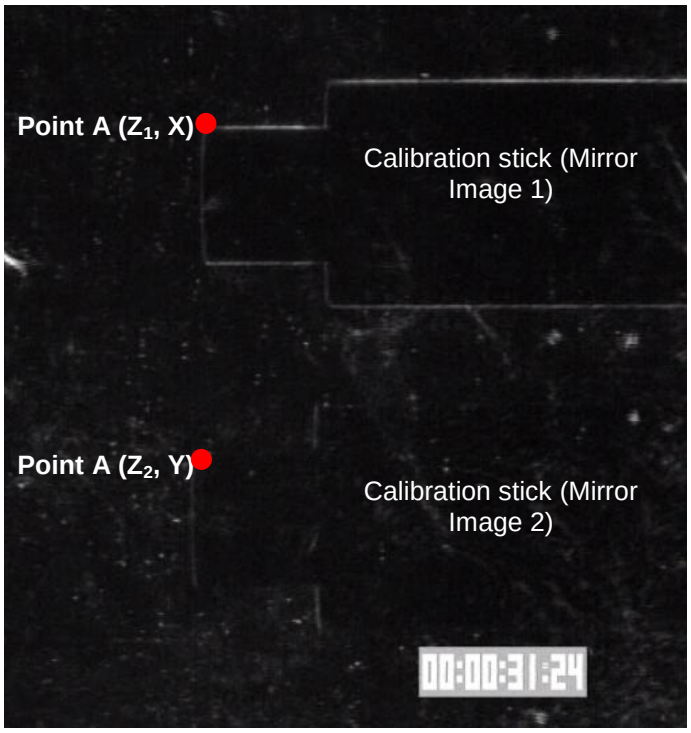


Figure 1.Obtaining 3D Tracks. Example image from Schlieren showing the calibration stick. X, Y, and Z coordinates were taken from the mirror images, as shown in Point A.

The first metric used to analyze the 3D trajectories was average swimming speed. Average swimming speed was determined by averaging the instantaneous swimming speeds (*t* =

0.033 seconds, 1 frame) across a single track. The instantaneous speed is defined as the distance traveled in the X, Y, and Z directions over a given time *t.*

$$V = \sqrt{(x_{t+1} - x_t)^2 + (y_{t+1} - y_t)^2 + (z_{t+1} - z_t)^2}$$

Tracks lasting ≤ 10 seconds were used for determination of average swimming speed to accurately represent behaviors that occur rapidly, including jumps. All average swimming speeds are reported as *mean* ± *standard error.* The net-to-gross-displacement ratio (NGDR) values of each track were also used to characterize swimming behavior. NGDR is the ratio between the gross distance travel and the net distance traveled, and describes the degree of path tortuosity, or the amount of turning present. A NGDR value of 0 is characteristic of diffuse swimming behavior, while a value of 1 characterizes ballistic swimming behavior. Only tracks lasting exactly 10 seconds were used in the calculation of NGDR values to account for the scale-dependency of the metric.

The swimming behavior "jump" was observed in some treatments. A jump is defined as an instantaneous speed value (averaged over three points/frames to eliminate noise remaining from tracking programs) of ≥ 1.0 cm/sec, a value determined by the distribution of instantaneous speed values across all trajectories and representing greater than three times increase in normal swimming speed. The frequency of jumps per track were counted and averaged within treatments for comparison.

Statistical Analysis. At least 10 tracks were collected from each experimental hour per replicate, and four replicates of each treatment were performed (see Table 3). Comparison of average swimming speeds and NGDR values were performed using the non-parametric Kruskal-Wallis (K-W) test due to the non-normal distribution of the data, as determined by the G-Test of Normality (Mahjoub et al 2011). The Wilcoxon Rank-Sum test was used to compare pairs when

a difference was detected, using the Bonferroni correction when multiple pair wise comparisons were performed. All statistical tests were performed using the R software package.

Table 3. Three-dimensional trajectories collected in each experimental hour by treatment

Hour	**Treatment**	**Total Number of tracks**
Treatment hour 1 (T1)	*R. lens* (100%)	53
	R. lens (20%)	41
	A. fundyense	55
	K. brevis	48
Depuration hour 0 (D0)	*R. lens* (100%)	72
	R. lens (20%)	43
	A. fundyense	77
	K. brevis	63
Depuration hour 15 (D15)	*R. lens* (100%)	66
	R. lens (20%)	40
	A. fundyense	61
	K. brevis	58

CHAPTER 3

RESULTS

Swimming Speed. The *R. lens* (100%) and *R. lens* (20%) controls were not significantly different with regards to swimming speed in any of the time periods ($p>0.05$, Wilcoxon rank-sum test) and were therefore combined into a single *R. lens* control. When *T. longicornis* is exposed to the allopatric *K. brevis* treatment the average swimming speed was immediately and significantly reduced compared to the *R. lens* control in Treatment hour 1 ($p=0.002$) and Depuration hour 1 ($p=0.001$), although the speed returned to control levels in Depuration hour 15 (Figure 2). In contrast, *T. longicornis* exposed to the sympatric *A. fundyense* treatment showed no significant response related to swimming speed during Treatment hour 1 and Depuration hour 0; however, a significant increase in swimming speed ($p=0.005$) compared to the *R. lens* control occurred in Depuration hour 15 (Figure 2). Patterns of swimming speed over time showed differences depending on the treatment. Copepods in the *K. brevis* treatment maintained a fairly consistent average swimming speed across Treatment hour 1 and Depuration hour 0 and 15 (Figure 2). Individuals in the *R. lens* control maintained similar average swimming speeds across Treatment hour 1 and Depuration hour 0, and showed a swimming speed decrease in Depuration hour 15 (Figure 2). *Temora longicornis* individuals in the *A. fundyense* treatment followed a similar pattern to the *R. lens* control over Depuration hours 0 and 15, although average speeds were higher (Figure 2). Animals demonstrated an increase in swimming speed from Treatment hour 1 to Depuration hour 0; however, the higher speed in Depuration hour 0 was not significantly differentthan the *R. lens* control. The lack of significance may be due to an accompanying increase in variance.

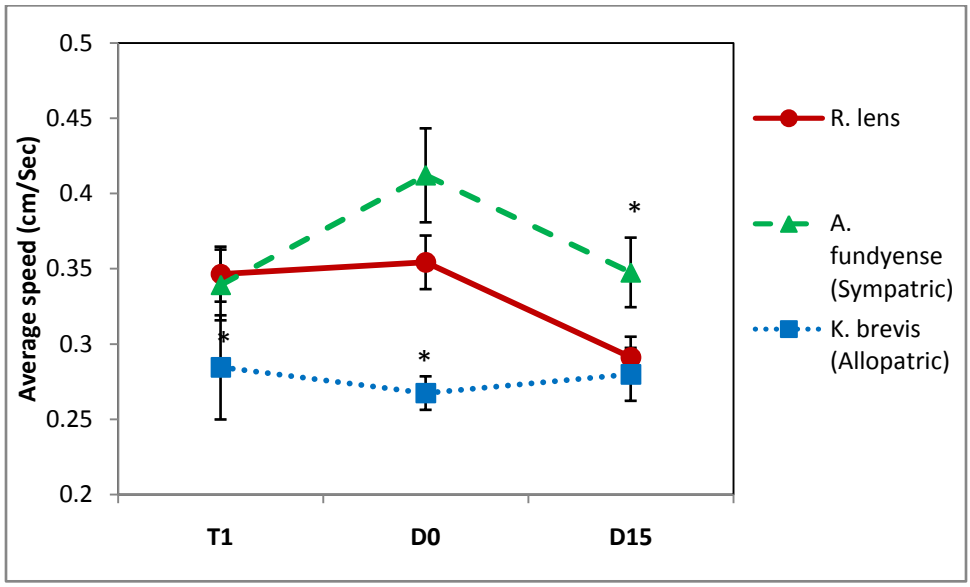


Figure 2 Exposure to allopatric HABs immediately impacts swimming speed. *T. longicornis* exhibited significantly decreased average swimming speeds compared to the *R. lens* control in Treatment hour 1 (0.284 ± 0.034 cm/sec, p = 0.002, Wilcoxon rank-sum test) and Depuration hour 0 (0.267 ± 0.011 cm/sec, p = 0.001, Wilcoxon rank-sum test) of the *K. brevis* treatment. In Depuration hour 15 after exposure to the *A. fundyense* treatment, the animals exhibited a significant increase in swimming speed compared to the *R. lens* control (0.348 ± 0.023 cm/sec, p=0.005, Wilcoxon rank-sum test). The Kruskal-Wallis test showed significant differences between treatments in each time period ($p<0.05$ in all time periods).

Path Tortuosity. As with swimming speed, the *R. lens* (100%) and *R. lens* (20%) controls were not significantly different during Treatment hour 1 and Depuration hour 0 ($p>0.05$, Wilcoxon rank-sum test) and were therefore combined into a single *R. lens* control. However, the two *R. lens* control treatments were significantly different during Depuration hour 15, with the *R. lens* control (20%) treatment showing a significantly higher average NGDR value than the *R. lens* control (100%) (p=0.002, Wilcoxon rank-sum test, Figure 3).

Temora longicornis exposed to the allopatric *K. brevis* treatment showed significantly reduced NGDR values compared to the *R. lens* control during Treatment hour 1 (p=0.0004), and this pattern persisted in Depuration hour 0 although significance was lost (Figure 3). *Temora*

longicornis exposed to the sympatric *A. fundyense* treatment showed no significant change from the *R. lens* control until Depuration hour 15 (Figure 3). Animals removed from the *A. fundyense* treatment showed significantly higher NGDR values than the high food concentration *R. lens* control (100%) (p=0.004); however, NGDR values were not significantly different from the reduced *R. lens*control (20%) (p=0.69). The scatterplot of swimming trajectories while in Treatment hour 1 of the *K. brevis* treatment and *R. lens* control show the clear differences in swimming path tortuosity (Figure 4). Swimming trajectories during Treatment hour 1 of the *K. brevis* treatment are more diffuse and cover shorter distances (Figure 4A), while trajectories during Treatment hour 1 of the *R. lens* control are longer and more ballistic, covering longer distances (Figure 4B).

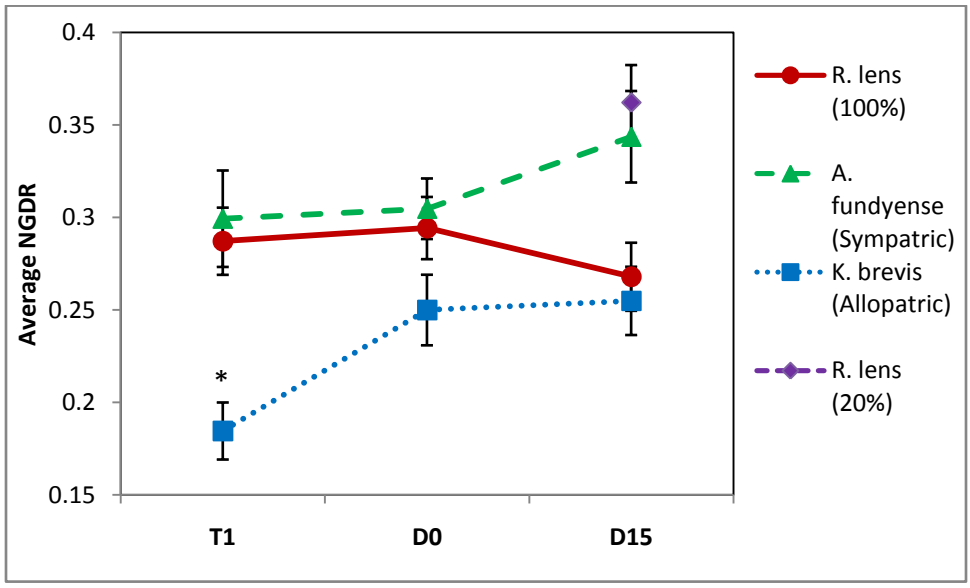
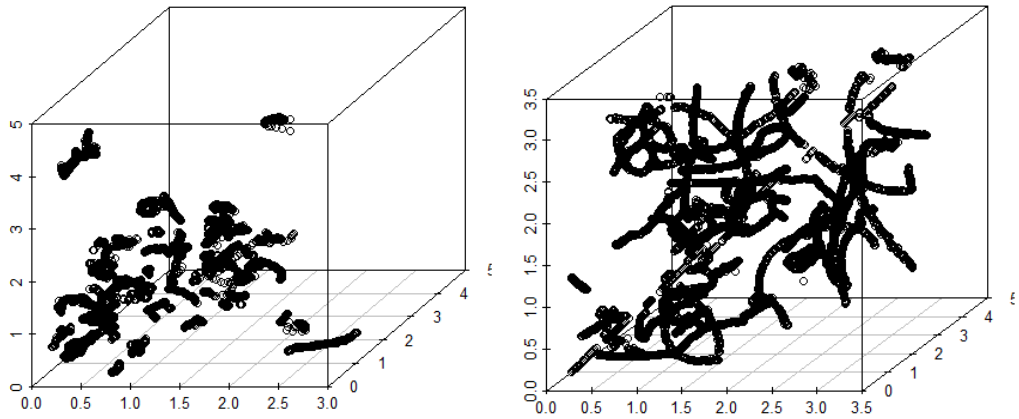


Figure 3.Difference exist in average net to gross displacement ratio in HAB treatments and controls. *T. longicornis* exposed to the *K. brevis* treatment showed a significantly decreased NGDR value than the *R. lens* control during Treatment hour 1 (0.184 ± 0.015, p=0.0004, Wilcoxon rank-sum test). Individuals exposed to *A. fundyense* showed a significantly greater NGDR than the *R. lens* control (100%) during Depuration hour 15 (0.344 ± 0.025, p = 0.004, Wilcoxon rank-sum test); however, the NGDR values were not significantly different from those in the *R. lens* control (100%) during Depuration hour 15 (p=0.69, Wilcoxon-rank sum test). The Kruskal-Wallis test showed significant differences between treatments during Treatment hour 1 and Depuration hour 15 ($p<0.01$ in treatment, $p>0.05$ for Depuration hour 0, and $p<0.01$ 15-16 hours after removal from treatment).

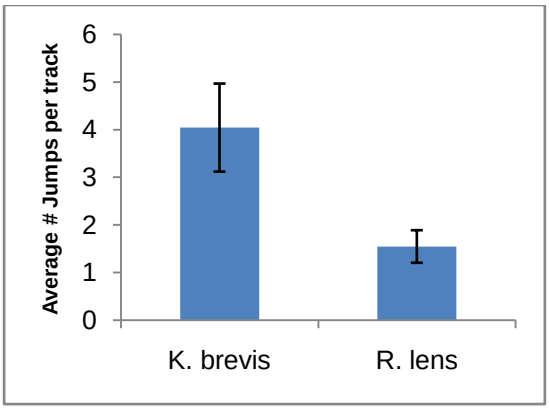


A. During *K. brevis* treatment **B. During *R. lens* treatment.**

Figure 4 Scatterplots of 3D swimming trajectories during Treatment hour 1 show differences in swimming behavior. A. *Temora longicornis* exhibited decreased swimming speeds and NGDR values during Treatment hour 1 of the *K. brevis* treatment, as is reflected in the reduced length and increase tortuosity of the trajectories. B. The individuals in Treatment hour 1 of the *R. lens* control exhibited higher average speeds and NGDR values, reflected in the longer, straighter trajectories. All axes in cm.

Swimming Behavior: Jumps. When *T. longicornis* is exposed to the *K. brevis* treatment, an immediate and significant increase in the behavior classified as "jumps" observed compared to those exposed to the *R. lens* control during Treatment hour 1 (Figure 5A, p=0.02, Wilcoxon rank-sum test). Plots of the instantaneous speed values over time visualize the distinct jumping behavior and show the differences between tracks of copepods exposed to the allopatric *K. brevis* and palatable *R. lens* (Figure 5B). The jumping behavior frequency was not different in Depuration hours 0 and 15 across all treatments.

A.



B.

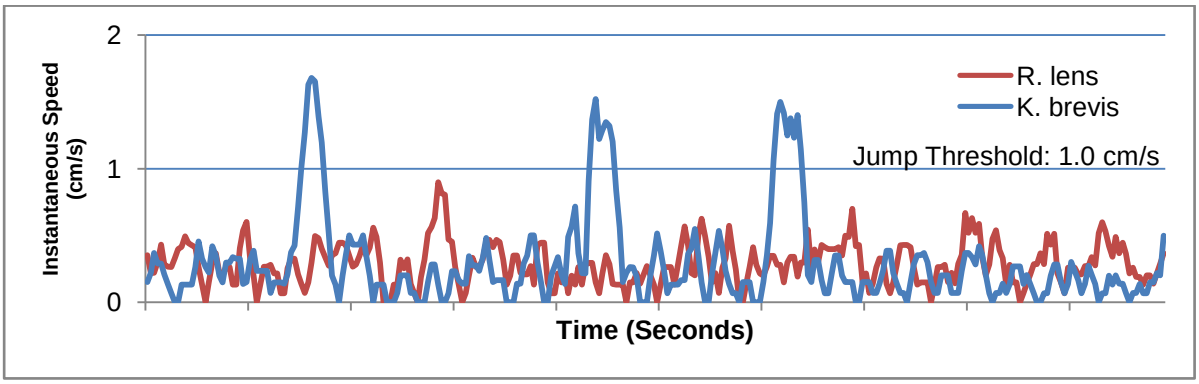


Figure 5Copepods jump more frequently in the *K. brevis* treatment during Treatment hour 1.A. Average number of jumps/accelerations per individual track during bloom exposure. The copepods swimming in Treatment hour 1 in the *K. brevis* treatment showed a statistically significant ($p = 0.02$) increase in jumps compared to those in the *R. lens* control (*K. brevis* treatment average = 4.04 ± 0.92, n = 48; *R. lens* control average = 1.54 ± 0.34, n = 90). **B.** Plot of instantaneous speed (cm/sec) over time (sec) of a representative track from *K. brevis* treatment and *R. lens* control in Treatment hour 1.

CHAPTER 4

DISCUSSION AND CONCLUSION

Sympatry vs. Allopatry and the Consequences on Kinematic Behavior. *Temora longicornis* individuals exposed to the sympatric and allopatric HAB species exhibited different behavioral responses, each suggesting different fitness consequences for both copepod and algae. Individuals exposed to the *A. fundyense* treatment exhibited behavior changes significantly different from the *R. lens* controls only after the 15-16 hour depuration period. Meanwhile, *T. longicornis* exposed to the *K. brevis* treatment exhibited immediate behavior changes during exposure (Treatment hour 1) compared to the *R. lens* control, and these effects lost significance during the depuration period.

The elevated swimming speed exhibited in Depuration hour 15 may increase copepod encounter rates with palatable food sources and predators, potentially increasing predator consumption of copepods (Visser and Kiorboe 2006, Lasley-Rasher 2012). The elevated swimming speed also could remove copepods from the bloom area more rapidly than those in a palatable environment of low or high food concentrations. The increase in copepod death due to predation and the removal of animals from the bloom environment are both potentially beneficial to *A. fundyense* bloom formation and growth through the reduction of grazing pressure. As other copepod species that co-occur with *A. fundyense* selectively screen food choices, preferring those with the lowest amount of saxitoxin, the combination of swimming and feeding behavior changes may act together to further reduce the grazing pressure on *A. fundyense* (Teegarden 1999). The changes in swimming speed may be due to the saxitoxin or another uncharacterized chemical produced by *A.* fundyense, but further research is required to determine the exact cause

of the observed behavioral differences.

While copepods in Depuration hour 15 swam faster than copepods in both *R. lens* controls (100% and 20%), animals only exhibited a higher NGDR value than the full biovolume *R. lens* control (100%). The animals in the reduced concentration *R. lens* control (20%) also exhibited higher NGDR values than the *R. lens* control (100%) and *K. brevis* treatment during Depuration hour 15. The similarity between the NGDR values in the *A. fundyense* treatment and *R. lens* control (20%) may be due to the low nutritional quality of *A. fundyense* (Teegarden 1999). *Temora longicornis* is known to consume *A. fundyense* at similar rates to *R. lens* despite the low nutrient levels, while the copepod species *A. tonsa* consumes *A. fundyense* at a significantly higher rate than palatable food sources to combat the reduced nutrient load (Lasley-Rasher 2012, Teegarden 1999). Copepods also exhibited ballistic trajectories in response to starvation conditions, a behavior change thought to increase the likelihood of encountering higher quality food sources (van Duren and Videler 1996). In this study, consumption rates were not measured during the behavior experiments, so the effect of nutrition on copepod behavior cannot be isolated from other factors. The increased NGDR value in the *A. fundyense* Depuration hour 15 also could be explained by reactions to the saxitoxin or other chemicals present in the water due to the presence of the HAB species. The increased NGDR values in the *R. lens* control (20%) compared to the *R. lens* control (100%) may be due to the lack of adequate food, but a measure of consumption rates over a two hour period in both palatable algae concentrations is necessary to confirm this hypothesis.

The allopatric pairing of *T. longicornis* and *K. brevis* resulted in several immediate changes in swimming behavior. During Treatment hour 1, copepods in the *K. brevis* treatment had decreased swimming speed and NGDR values and an increased jump frequency in

comparison to the *R. lens* control. The lower swimming speed and NGDR values would limit copepod movement while exposed to a *K. brevis* bloom in the marine environment, resulting in continued exposure to the brevetoxins and other chemicals produced by the HAB species and a reduced contact rate with palatable food and predators. A *K. brevis* heavy diet decreases *T. longicornis* survivorship after four days to exposure compared to all other diet treatments, including *R. lens*, *A. fundyense*, and a starvation treatment (Lasley-Rasher et al. unpublished data). The behavior changes induced by the *K. brevis* treatment could lead to an increase in copepod death over the lifetime of a bloom, which can last for months in the field (Anderson 1994, 1997). While the immediate consequence of the limited movement may increase local feeding on *K. brevis*, the benefit of decreased survivorship may outweigh the initial cost associated with the grazing period. *Temora longicornis* is known to consume *K. brevis* at rates similar to *R. lens*, but the grazing intensity was not monitored during these behavior experiments (Lasley-Rasher et al. unpublished data). Further research into the long-term impact of copepod grazing on a *K. brevis* diet would illuminate the impact of *K. brevis* on *T. longicornis* fitness and the impact of sustained *T. longicornis* grazing on a population of *K. brevis.*

The increase in jump frequency exhibited by copepods during *K. brevis* Treatment hour 1adds another facet to the behavioral responses induced by allopatric HABs. Jump behavior may be caused by a reaction to the brevetoxins or other chemicals produced by *K. brevis*. Brevetoxins bind to the sodium channels in nerve cells, which can lead to the disruption of natural neurological signaling processes (Watkins et al. 2008). Although studied more extensively in vertebrate systems, the behavioral changes seen in *T. longicornis* could be explained by neurological disruption, particularly as jumps are no longer observed at an increased frequency during the depuration period (Watkins et al. 2008). Further research

concerning brevetoxins and other neurotoxins on the invertebrate nervous system are needed to determine the exact physiological effects of HAB chemicals.

Jumps are a disruptive behavior, which may increase copepod visibility to visual predators and those relying on hydrodynamic cues (Buskey 1994). As jumps only cover a distance of a few body lengths per occurrence, it is unlikely that this behavioral change would remove *T. longicornis* completely from *K. brevis* blooms in the marine environment, although it may allow movement between patches of different HAB concentrations. The increase in visibility caused by jumps may increase the predation rate on copepods, reducing the total number in the bloom environment. With fewer grazers, *K. brevis* could continue to grow at an increased rate, forming and maintaining the high concentrations necessary to form expansive blooms. However, the reduced swimming speed and diffuse swimming paths would decrease the overall encounter rate with predators, which may counter the increased visibility due to jumps (Visser and Kiorboe 2006, Buskey 1994). An updated encounter rate model that integrates short term behavioral events with long-term swimming trends is needed to determine the combined impact of the two behavioral changes.

The Potential for Invasion. *Karenia brevis* blooms occur yearly in the Gulf of Mexico, and are colloquially called Florida Red Tides due to their prevalence along Florida's gulf coast (Watkins et al. 2008). However, blooms have occurred farther north along the Atlantic coast, with one lasting for four months off the coast of North Carolina in 1987 (Tester et al. 1991). As the geographical range of HABs expand, whether due to changes in ocean currents, temperature, or nutrient load, understanding the interaction between allopatric HAB species and zooplankton grazers becomes an important research topic (Roelke and Buyukates 2001). Zooplankton are pivotal in oceanic food webs, and disruption to copepod populations through behavioral and

physiological changes would not only impact HAB growth, but cause a cascading effect up the food chain, affecting fish, oceanic mammals, and humans (Turner 2004). A more complete understanding of the impact of allopatric HABs on copepods could increase our ability to predict the impact of HAB invasions when they occur, perhaps allowing for partial amelioration of the negative effects.

Arms Race: HAB and Copepod Co-evolution. Populations of *A. hudsonica* with historical exposure to *A. fundyense* exhibited tolerance to the sublethal fitness effects seen in naive populations (Colin and Dam 2005). The results from these behavior experiments add support to the hypothesis that historical exposure leads to increased HAB resistance. *Temora longicornis* individuals showed no measurable behavioral reaction to sympatric *A. fundyense* during Treatment hour 1 and Depuration hour 0. However, when exposed to the allopatric *K. brevis*, behavior changes occurred only in Treatment hour 1 and Depuration hour 0. The immediate reaction observed in the allopatric pairing suggests that the *T. longicornis* individuals were impacted by the neurotoxins or other chemicals produced by the allopatric HAB species. Exposure to novel HAB species has multiple negative fitness and behavioral consequences on the zooplankton grazers (Colin and Dam 2005, Turner 2012). These negative effects may release HABs from predator control, allowing large-scale growth and the formation of blooms. Over time, selective pressure by the HAB species causes population demographics to shift, where only copepods with inherent resistance to these chemicals survive and reproduce, eventually leading to a HAB-resistance population (Colin and Dam 2005). The *T. longicornis* from the Gulf of Maine, having experienced *A. fundyense* blooms on an almost yearly basis, exhibit this resistance, as seen by the lack of sublethal and immediate behavioral effects during HAB exposure (Anderson 2005).

As zooplankton population resistance increases, the HAB species may experience an increase in grazing pressure. The grazing pressure may in turn shift algal population demographics, where the only individuals that thrive are those that induce additional physiological or behavioral reactions in the zooplankton predators. *Alexandrium fundyense* and *K. brevis* have low nutrient loads, inducing zooplankton predators to selectively prefer alternate algal food sources (Teegarden 1999, Prince et al 2006). The behavioral changes seen in *T. longicornis* in Depuration hour 15 after the *A. fundyense* treatment supports this hypothesis as well. Through production of saxitoxins, other chemicals, or reduction of nutrients, *A. fundyense* exposure causes a behavioral response in copepods that may result in increased encounter rates with predators and drive zooplankton out of the bloom. Both behavioral consequences may reduce copepod populations, and are beneficial to *A. fundyense* fitness and the formation of blooms.

Carrying these results forward, populations of *T. longicornis* in the Gulf of Maine may adapt to *A. fundyense* further, eventually eliminating the observed behavioral changes and regaining the upper hand in the arms race. However, *A. fundyense* populations also will continue to evolve and develop new strategies to avoid predation. Predator and prey will continue to co-evolve, constantly developing new and innovative strategies for survival.

A copepod bioassay: red tide warning system. The study of behavior, even in small planktonic crustaceans, is important to biological research due to the immediate, observable results. As demonstrated in this study, *T. longicornis* react immediately when exposed to the equivalent of a medium-concentration *K. brevis* bloom. In particular, the increase in jumps is highly visible, even to the untrained observer. Stock cultures of *T. longicornis* can be combined with water samples suspected of containing *K. brevis* and observed. If an increase in jump

frequency occurs, the conclusion can be reached that *K. brevis* is likely in the water, and alerts sent out to neighboring fisheries and beaches.

Additional experiments testing *T. longicornis* sensitivity to *K. brevis* would increase the power of the behavioral bioassay. By exposing *T. longicornis* individuals to *K. brevis* at various concentrations, the minimum algal load required to cause a behavioral reaction also canbe determined. Depending on the threshold algal concentration, the copepod bioassay could be used as an early warning system, detecting *K. brevis* before the formation of a large, damaging bloom. As research into the mechanisms fueling HABs continue, presence of a reliable and simple early warning system may help reduce damage to marine ecosystems and fisheries and prevent public health issues due to the presence of neurotoxins. The recent deaths of >10% of Florida's manatee population may have been lessened by detecting *K. brevis* early (Wines 2013). Preventing damage and disease could save coastal states millions of dollars in lost revenue and health care costs, as well as protect coastal ecosystems from the devastation of large, long lasting HABs (Anderson et al. 2010).

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